

Attorney Docket No. 015837-0280633

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

James Robl et al.

Group Art Unit: 1632

Application No. 09/874,040

Examiner: Anne Marie Sabrine Wehbe

Filed: June 6, 2001

Title: EMBRYONIC OR STEM-LIKE CELL LINES PRODUCED BY CROSS
SPECIES NUCLEAR TRANSPLANTATION

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DECLARATION PURSUANT TO 37 C.F.R. § 1.132Hon. Commissioner of Patents
Washington, D.C. 20231

Sir:

I, Robert P. Lanza, M.D., declare and state as follows:

(1) I have been employed by Advanced Cell Technology, as Vice-President of Medical & Scientific Development, since March 15, 1999.

(2) I am a former Fulbright Scholar, have been nominated for a MacArthur Foundation award, and have over 200 scientific publications, books, and patents, including "Principles of Cloning," "Cloning of an Endangered Species (*Bos Gaurus*) Using Interspecies Nuclear Transfer," "Generation Of Histocompatible Tissues Using Nuclear Transplantation," "Principles of Tissue Engineering," and "Medical Science and the Advancement of World Health."

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(3) Previously, I worked from 1990 to 1998 at BioHybrid Technologies, wherein I conducted and directed research in the areas of tissue engineering and cell and tissue (cross-species) transplantation.

(4) I have published numerous articles relating to cloning, including interspecies nuclear transfer, I am a co-inventor of pending patent applications relating to intra- and interspecies cloning, and I hold several patents relating to transplantation medicine. My expertise in the art is further established by the attached curriculum vitae.

(5) I have reviewed the Office Actions dated December 19, 2001, and September 10, 2002, including the rejection of claims 36-87 under 35 U.S.C. § 103(a) as being unpatentable over Wolfe et al., in view of Collas et al. It is my understanding that the Examiner believes that the combination of the disclosures of the Wolfe et al. and Collas et al. references, taken as a whole, would have suggested the invention of claims 36-87 to a person of ordinary skill in the art. For the reasons discussed below, I respectfully disagree.

The present application is a continuation of U.S. Patent Application No. 08/699,040, which was filed on August 19, 1996. In 1996, when the parent application was filed, the inventors' discovery that the nucleus of a differentiated human cell could be transferred into an enucleated bovine oocyte to produce a nuclear transfer embryo capable of undergoing cleavage and developing into a multicellular structure was an unexpected and surprising result. To my knowledge, the disclosure in the present application is the first demonstration of the successful production of a multicellular embryo by inter-specific nuclear transfer using a differentiated nuclear donor cell.

In the late 1980s and early 1990s, methods were developed for cloning by nuclear transfer that used undifferentiated embryonic cells, i.e., blastomeres, as nuclear donor cells.

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At that time, it was recognized that the development of a fertilized oocyte and formation of a blastocyst involves many complex interactions between the nuclear chromatin and factors in the recipient oocyte. These interactions were known to involve chemical modification, removal, and addition of histone and non-histone chromatin proteins, one result of which is the activation of transcription of genes of the embryonic genome (Nothias et al., 1995, J. Biol. Chem., 270:22077-80). At that time, it was the generally held view of persons in the field that as donor embryonic cells differentiated, they lost their capacity to direct embryonic development and blastocyst formation. As discussed in the cited Collas et al. reference, the molecular basis for the restricted developmental potential of nuclei from differentiated cells was not known, but was thought to be due, at least in part, to changes related to DNA replication and gene expression. For example, it was proposed that incomplete DNA replication could result from asynchrony in the cell cycle of the donor nucleus and recipient oocyte, or from changes in the pattern of transcriptionally active and inactive chromatin and their timing of replication. Failure to reactivate genes that became transcriptionally inactive during differentiation was also proposed as a reason for the apparent inability of the nucleus of a differentiated cell to direct embryonic development following transfer into an enucleated oocyte (see Collas et al., p. 266).

Similar reactions and protein exchanges between the recipient ooplasm and the donor chromatin were also thought to be necessary in order to "remodel" or "reprogram" a nucleus of a differentiated cell that is transferred into an oocyte so that it is capable of successfully directing embryogenesis (for example, see Prather et al., J. Reprod. Fertil. Suppl., 1990, 41:125-34). However, it was recognized that reprogramming following inter-specific nuclear transfer was even more complex and unpredictable than reprogramming associated with intra-specific nuclear transfer, because all of the complex reactions and interactions between the nuclear chromatin and components of a recipient oocyte that are required for embryonic

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development would have to occur across the evolutionary divide between the two species. For example, it was recognized that evolutionary divergence of the structures of the nuclear chromatin relative to the corresponding oocyte components could result in structural incompatibilities that inhibit or alter reactions required for successful embryogenesis. Species-specific variations in the timing of synthesis or degradation and/or in the concentrations of chromatin binding sites, oocyte components, other structural proteins, and metabolic enzymes, were seen as adding an additional layer of complexity to embryonic development following inter-specific nuclear transfer. At the time the parent application was filed, it was known that the complement of proteins that is initially produced by an embryonic genome varies from species to species (Crosby et al., *J. Reprod. Fertility*, 1988, 82:769-75; Barnes et al., *Molecular Reproduction and Development*, 1991, 29:117-9; and Connover et al., *Dev. Biology*, 1991, 147:403-14). In addition, it was known that the stage of embryonic development at which transcription of embryonic genes begins, referred to as the "maternal to embryonic transition" (MET), also varies significantly from species to species. For example, transcription of genomic DNA begins in murine and rat embryos at the 2-cell stage, in bovine embryos at the late 4- to early 8-cell stage, in human embryos at the 4- to 8-cell stage, and in sheep embryos at the 8- to 16-cell stage (reviewed by Telford et al., *Molecular Reproduction and Development*, 1990, 26:90-100). Species-specific incompatibilities between the oocyte-derived mitochondria and proteins expressed by nuclear genes were seen as another type of species-specific discordance that could interfere with the successful development of embryos produced by cross-species nuclear transfer. Mammalian mitochondrial DNA codes for 13 enzymes that mediate oxidative phosphorylation, 22 tRNAs, and two rRNAs (Smith et al., *J. Reprod. Fertil. Suppl.* 48:31-43, 1993). Kenyon et al. showed that oxidative phosphorylation is impaired in cells with human genomic DNA and mitochondria of orangutan, New World monkeys, or lemurs, but not in cells with human

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genomic DNA and mitochondria of gorillas or chimpanzees (Proc. Nat. Acad. Sci. U.S.A., 94:9131-9135, 1997).

In view of the many uncertainties associated with inter-specific nuclear transfer, the report by Wolfe et al. that blastocysts could be generated following inter-specific transfer of blastomere nuclei into recipient oocytes was initially regarded as significant, because it suggested that evolutionary divergence does not prevent interactions between an undifferentiated nucleus of one species and the ooplasm of a different species that mediate successful development of a nuclear transfer embryo. However, an article by Wolfe et al. (Biol. of Reprod., 1994, 50 (Suppl. 1) p. 72) that was written by three of the same authors as the Wolfe et al. (1990) reference that was cited as prior art, raised doubts as to whether the 1990 reference actually demonstrated production of a blastocyst directed by a nucleus of one species after transfer into an oocyte of a different species. The 1994 Wolfe et al. reference stated that the nuclear transfer methods used to obtain the results reported in the 1990 reference did not include checking to verify that the recipient oocyte was successfully enucleated, and it concluded that the blastocysts reported in the 1990 reference as having been obtained by interspecific nuclear transfer may have actually been the result of parthenogenetic development of nucleated demi-oocytes. The 1994 Wolfe et al. reference further reported that repetition of the caprine/bovine interspecific nuclear transfer experiments using a method that included verification of enucleation did not result in production of blastocysts following interspecific nuclear transfer, and concluded that the interspecies nuclear transfers were incapable of developing into blastocysts. In view of the statements in the 1994 Wolfe et al. reference regarding the results reported in the 1990 Wolfe et al. reference, persons of ordinary skill in art would not have reasonably regarded the 1990 Wolfe et al. reference as suggesting that interspecific nuclear transfer would result in successful embryonic development.

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The 1990 Wolfe et al. reference described performing interspecific nuclear transfer using donor nuclei of undifferentiated blastomeres; therefore, even if such a method had been capable of producing a blastocyst, it was qualitatively different from the claimed invention, which performs inter-specific nuclear transfer using donor nuclei of differentiated cells. As discussed above, nuclear transfer using undifferentiated donor nuclei was a known and routinely practiced cloning technique at the time the parent application was filed. Persons skilled in the art knew that the chromatin of embryonic donor cells is in an embryonic configuration that is capable of directing embryogenesis with relatively high efficiency. In contrast, successful cloning by nuclear transfer using donor nuclei of differentiated cells was known to be a relatively new and much more problematic methodology. Persons skilled in the art knew that the chromosomes of a differentiated donor cell are in a non-embryonic state, and must undergo significant reprogramming in order for successful embryonic development to occur. The first report of cloning by somatic cell nuclear transfer that resulted in the development and birth of a live mammal was published in March 1996 (Campbell et al., Nature, 1996, 38:64-66). The authors of that report hypothesized that their surprising finding of successful reprogramming was facilitated to some extent by chromatin structures associated with quiescent donor cells (p. 66). Within a year after Campbell et al. reported their success in cloning by somatic cell nuclear transfer using quiescent donor cells, scientists of Advanced Cell Technology, the assignee of the present application, successfully cloned bovines by somatic cell nuclear transfer using non-quiescent donor cell nuclei (U.S. Patent No. 5,945,577). The molecular basis for reprogramming a differentiated cell nucleus following nuclear transfer into a recipient oocyte remains poorly understood to this day. As discussed above, successful reprogramming of a differentiated donor cell nucleus in an oocyte of a different species was seen as involving the complex reactions and interactions between the chromatin of the differentiated cell and oocyte components that are required for

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reprogramming a nucleus of like species, plus additional, species-specific and time-sensitive reactions and interactions between the donor chromatin and the components of the recipient oocyte that are unique to reprogramming a nucleus following inter-specific transfer. In fact, in view of the combined effects of structural incompatibilities between the proteins of the nuclear chromatin and oocyte components that could interfere with reprogramming, together with species-dependent asynchronies in the timing and pattern of embryonic gene expression, and discordance between genome- and oocyte-derived mitochondrial proteins, persons of ordinary skill in the art at the time the parent application was filed could not have predicted whether or not interspecies nuclear transfer using differentiated donor cell nuclei would lead to successful embryonic development and production of blastocysts. Accordingly, even if the 1990 Wolfe et al. reference had convincingly described successful embryonic development following inter-specific nuclear transfer using donor nuclei of undifferentiated blastomeres, this result would not have suggested to a person of ordinary skill in the art that inter-specific nuclear transfer using donor nuclei of differentiated cells would reasonably be expected to result in embryonic development.

The Collas et al. reference showed that a blastocyst could be obtained following transfer of a nucleus of a differentiated somatic cell into an enucleated oocyte of the same species; but the Collas et al. reference, in combination with the 1990 Wolfe et al. reference, would not have suggested to a person of ordinary skill in the art that inter-specific nuclear transfer using differentiated donor cell nuclei would lead to successful embryonic development. In the first place, the 1994 Wolfe et al. reference stated that the methodology of the 1990 Wolfe et al. reference was flawed, and that attempts to reproduce the production of blastocysts by inter-specific nuclear transfer were unsuccessful, so that one of ordinary skill in the art would not have relied on the teachings of the 1990 Wolfe et al. reference, as discussed above.

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Secondly, at the time the parent application was filed, the process of reprogramming a differentiated donor nucleus following inter-specific nuclear transfer so that embryogenesis can proceed was regarded as involving complex, unpredictable interactions and reactions between the donor nuclei and oocytes and properly regulated transcription of genes of the embryonic genome, as discussed above. The Collas et al. reference only describes the reprogramming of a cell nucleus in an oocyte of the same species. In view of the additional complexities and reasons for uncertainty believed to be associated with reprogramming inter-specific nuclear transfer, the results reported by Collas et al., alone or in combination with Wolfe et al., would not have enabled a person of ordinary skill in the art to predict whether successful embryonic development would occur following transfer of the nucleus of a differentiated cell into an oocyte of a different species. Therefore, the successful operation of the claimed invention would not have been obvious to a person of ordinary skill in the art at the time the parent application was filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 3/10/03
Robert P. Lanza



CURRICULUM VITAE

Name: Robert Paul Lanza
Place of Birth: Boston, Massachusetts

Private Address: South Meadow Pond Island, 35 S. Meadow Road,
Clinton, Massachusetts 01510-4327

Work Address: Advanced Cell Technology, Inc.
One Innovation Drive, Worcester, Massachusetts 01605

Citizenship: United States

Education: University of Pennsylvania, M.D., 1983
University of Pennsylvania, B.A., 1978
(Fulbright Scholar, Benjamin Franklin Scholar,
University Scholar, Professor Howe Buck Scholar)

Professional Appointments:

2000-present Vice-President of Medical & Scientific Development
Advanced Cell Technology, Worcester, Massachusetts

1999-2000 Senior Director, Tissue Engineering & Transplantation
Medicine, and Vice-President of Business Development,
Advanced Cell Technology, Worcester, Massachusetts

1993-1998 Director, Transplantation Biology, BioHybrid Technologies
Inc., Shrewsbury, Massachusetts

1994-1995 Clinical Associate Professor of Surgery, Tufts University,
Grafton, Massachusetts

1991-1993 Associate in Surgery, Harvard Medical School, and the New
England Deaconess Hospital, Division of Organ
Transplantation, Boston, Massachusetts

1990-1993 Senior Scientist, BioHybrid Technologies Inc, Shrewsbury,
Massachusetts

Current Civic Appointments:

2001- Present Director, Clinton Greenway Conservation Trust
1998- Present Director, South Meadow Pond & Wildlife Association
1998- Present Conservation Commission, Town of Clinton, Massachusetts

Fellowships:

1988-1990 Mary K.Iacocca Transplantation Fellow, Departments of

Surgery and Medicine, UCLA School of Medicine, Los Angeles, California

1982-1984 Honorary Christiaan Barnard Research Fellow, Department of Cardiothoracic Surgery, University of Cape Town Medical School, Cape Town, Republic of South Africa

1978-1979 Fulbright Scholar/ITT International Fellow, and Visiting Scientist, Departments of Virology and Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel

Basic and Clinical Research Experience:

1988-1990 Departments of Surgery and Medicine, UCLA School of Medicine, and Affiliate, Pancreatic Transplant and Diabetes Research Laboratory, Wadsworth VA Medical Center, Los Angeles, California

1982-1984 Cardiac Surgical Research Unit and Department of Cardiothoracic Surgery, Groote Schuur Hospital and the University of Cape Town Medical School, Cape Town, Republic of South Africa (with Professor Christiaan Barnard, Emeritus Professor of Surgical Science). Professor Barnard performed the world's first human-heart transplant.

1979-1981 Department of Psychology and Social Relations, Harvard University, Cambridge, Massachusetts (with Professor B.F. Skinner, Edgar Pierce Professor of Psychology, Emeritus. Founder of modern behaviorism).

1978-1979 Departments of Virology and Chemical Immunology Weizmann Institute of Science, Rehovot, Israel

1978 Salk Institute for Biological Studies, San Diego, California (with Dr. Jonas Salk, Founder and Director of the Institute). Dr. Salk developed the polio vaccine.

1977 Department of Biochemistry, Oxford University, Oxford, England (with Professor Rodney Porter, recipient of the 1972 Nobel Prize in Medicine and Physiology)

1976-1977 Department of Anatomy, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania (with Dr. Howard Holtzer, Director of the Program in Cell Differentiation)

1976 Division of Molecular Biology, Rockefeller University, New York, New York (with Dr. Gerald Edelman, recipient of the 1972 Nobel Prize in Medicine and Physiology)

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| 1975-1976 | Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts (with Dr. Richard Hynes, Director of the Center) |
| 1974 | Department of Neurobiology, School of Medicine, Harvard University, Boston, Massachusetts (with Dr. Steven Kuffler, University Professor and Chairman of the Department) |
| 1973 | National Science Foundation/Pre-College Training Program, Jackson Laboratory, Bar Harbor, Maine |

PUBLISHED WORK

Articles in Journals and Magazines (and Presentations at Meetings):

Papers on Cloning and Stem Cells

Lanza, R.P., Arrow, K.J., Axelrod, J., Baltimore, D., Benacerraf, B., Bloch, K.E., Bloembergen, N., Brown, H.C., Brown, M.S., Cibelli, J.B., Cohen, S., Cooper, L.N., Corey, E.J., Dulbecco, R., Fischer, E.H., Fitch, V.L., Friedman, J.L., Friedman, M., Furchgott, R.F., Gell-Mann, M., Glaser, D.A., Glashow, S.L., Gilbert, W., Goldstein, J.L., Gould, S.J., Guillemin, R., Hauptman, H.A., Hershbach, D., Hoffman, R., Hood, L., Hubel, D.H., Karle, J., Klein, L.R., Kohn, W., Kornberg, A., Krebs, E.G., Lederman, L.M., Lederberg, J., Lee, D.M., Lucas, R.E., Marcus, R.A., Merrifield, R.B., Miller, M.H., Modigliani, Molina, M.J., Mullis, K., Murad, F., Murray, J.E., Nathans, D., Nirenberg, M.W., North, D.C., Olah, G.A., Palade, G.E., Perl, M.J., Ramsey, N.F., Richter, B., Robert, R.J., Robl, J.M., Samuelson, P.A., Schwartz, M., Sharp, P.A., Smalley, R.E., Smith, H.O., Solow, R.M., Taube, H., Tonegawa, S., Watson, J.D., Weinberg, S., Weller, T.H., West, M.D., Wieschaus, E.F., Wiesel, T.N., and Wilson, R.W.: Science over politics. Science 283:1849, 1999.

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Lanza, R.P., Caplan, A.L., Silver, L.M., Cibelli, J.B., West, M.D., and Green, R.: The ethical validity of using nuclear transfer in human transplantation. The Journal of the American Medical Association (JAMA), 284(24):3175, 2000.

Lanza, R.P.: Cloning and embryonic stem cells. 3rd Annual Meeting of the Genetics, Theology and Ethics Group, convened under the auspices of The Jesuit Institute at Boston College, Boston, Massachusetts, 1999.

Lanza, R.P.: Cloning and stem cells. The Wellcome Trust Science Policy Seminar (London, United Kingdom, 1999).

Lanza, R.P.: Human replacement therapy. Extended Life, Eternal Life: Biotechnological "Immortalization" - It's Scientific Basis, Future Prospects, and Ethical and Theological Significance Cohosted by The John Templeton Foundation and The Center for Bioethics, University of Pennsylvania, Philadelphia, Pennsylvania, 2000.

Lanza, R.P.: Current and future opportunities in stem cell research for organ regeneration. Royal Pharmaceutical Society and British Pharmaceutical Conference: Medicines - The Future Horizon, Birmingham, UK, 2000.

Lanza, R.P., Cibelli, J.B., Diaz, F., Moraes, C.T., Farin, P.W., Farin, C.E., Hammer, C.J., West, M.D. and Damiani, P. Cloning of an endangered species (Bos gaurus) using interspecies nuclear transfer. Cloning 2(2):79, 2000.

Lanza, R.P. Keynote Address: The use of cloning in human medicine, Public Symposium - Human Cloning: Bane or Blessing, sponsored by the University of Tennessee and the First United Methodist Church, Oak Ridge, Tennessee, 2000.

Lanza, R.P. Scientific overview. Human Genome Human Being Forum: Discovering the Human Context For Genetic Technologies, sponsored by The Global Academy, The Link Foundation, and Peking University (Beijing, China, 2000).

Lanza, R.P. Keynote Address: Stem cell based bio-engineering Tissue Engineering Symposium at the Canadian Chemical Engineering Conference (Montreal, Canada, 2000).

Lanza, R.P. Biodiversity and genetic technology. State of the World Forum / United Nations Millennium Conference (New York, New York, 2000). Included the largest assemblage of heads of state in history.

Lanza, R.P., Dresser, B.L., and Damiani, P. Cloning Noah's Ark: Biotechnology might offer the best way to keep some endangered species from disappearing from the planet. SCIENTIFIC AMERICAN 283(5): 84, 2000.

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Lanza, R.P., Silver, L.M., Cibelli, J.B., West, M.D., and Green, R.: Ethical issues in embryonic stem cell research. The Journal of the American Medical Association (JAMA), 285(11): 2001.

Lanza, R.P., and West, M.D. (Corresponding authors -- petition signed by 80 Nobel laureates urging President Bush to fund research on pluripotent stem cells): To the Honorable George W. Bush, President of the United States. The Washington Post, February 21, 2001.

Lanza, R.P.: Keynote Address: How tissue engineering and the new biology are shaping the future of organ replacement (47th Annual Conference of the American Society of Artificial Internal Organs (ASAIO), New York, New York, 2001).

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engineering. International eHealth Association, in association with the British Association for the Advancement of Science (London, UK 2002).

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Lanza, R.P. Embryonic stem cell research and cloning. Covering Stem Cell Research, Cloning, and Other Medical Advances. Foundation for American Communication (Cambridge, Massachusetts 2002)

Lanza, R.P. Keynote Address: Stem cell research, cloning, and the future of medicine. Brown University Distinguished Speakers Forum (Providence, Rhode Island 2002)

Lanza, R.P. Keynote Address: Nuclear transplantation (therapeutic cloning), stem cells and engineering of histocompatible organs in large animals. 2eme Congrès de la Société Francophone Transplantation: Organogenèse et Thérapie Cellulaire (Montréal, Québec, Canada 2002).

Lanza, R.P. Australian Museum's 175th Anniversary Lecture: The use of cloning in conservation and medicine (Sydney, Australia, 2002).

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Far? (Vail, Colorado 2002).

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International Society for Artificial Organs, Montreal, Canada, 1991).

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